Human Dermal Fibroblast Viability and Proliferation in vitro

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Objectives

- Determine effect of ethanol on Human Dermal Fibroblast (HDF) cells
- Develop relationship between absorbance and HDF cell concentration
- Assess effect of fetal bovine serum (FBS) on growth rate of HDF cells

Live/Dead Fluorescence Assay

- Seed cells in 24-well plate and incubate 2 days
- Add dye of 4 μM ethidium homodimer (EthD-1) and 2 μM calcein AM to each well
- Condition A: add 250 μL phosphate buffered saline (PBS)
- Condition B: add 250 μL ethanol
- Condition C: add 250 μL PBS, 2 drops ethanol
- View cell morphology using light microscope
- View cell color using fluorescent microscope

MTT Viability Test

- Seed 2 plates and incubate 2 days: cells counted on Coulter Counter, cells treated with MTT dye
- Seed wells at stock (50,000 cells/mL), 1:1.5, 1:2, 1:3, 1:6, 1:12 dilutions, and media control
- MTT dye plate: add 75 μL dye, incubate 2 hrs, add Solubilization/Stop solution, incubate 45 min
- Measure absorbance of sample from each MTT dye well at 570 nm on spectrophotometer
- Coulter Counter plate: find cell concentration of each well using Coulter Counter

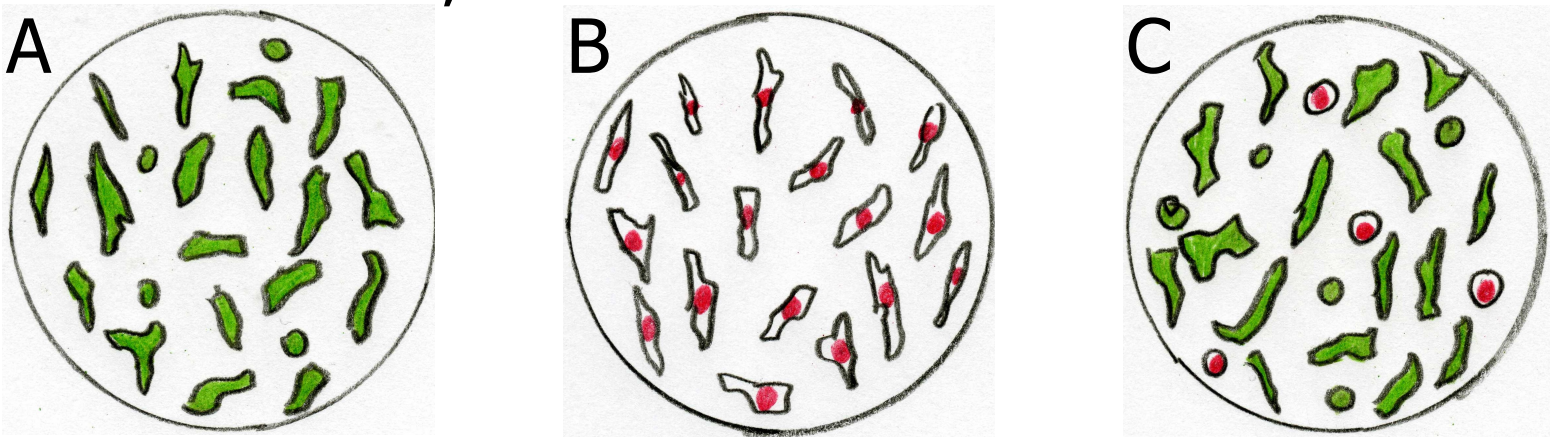
Cell Proliferation Assay

- Seed 33 wells at 5,000 cells/mL in DMEM with 1% serum and incubate 4 hrs
- 4 hrs: count cell number of 6 wells using Coulter Counter
- 4 hrs: replace media in 9 wells with DMEM with 1%, 5% and 10% serum, respectively
- Day 2, 5, 7: count cell number of 3 wells of each % serum at each time point
- Day 2, 5: replenish media for remaining wells

Ethanol Kills HDF cells

Observations from light and fluorescent microscopes

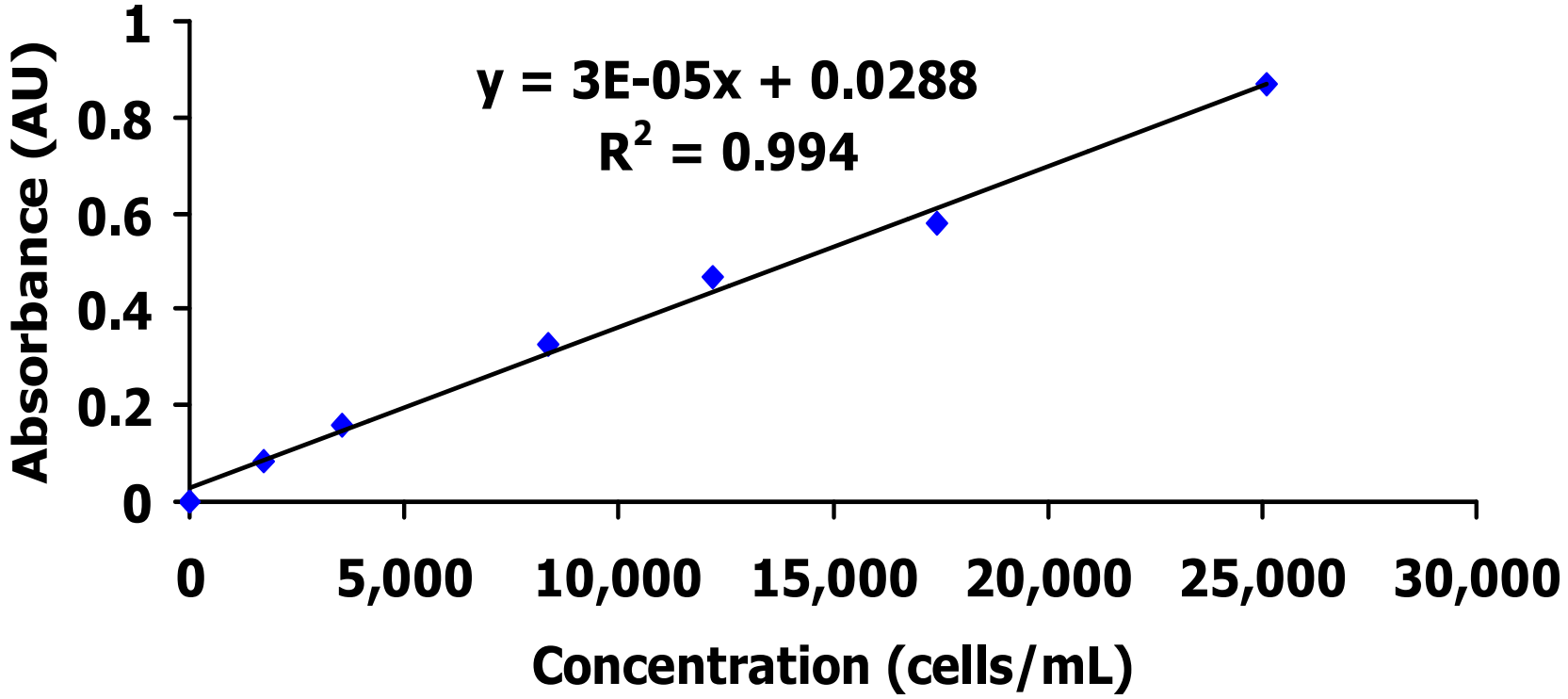
Green = alive; Red = dead



- A: 100% of cells in PBS only were alive
- B: 100% of cells in 250 μL ethanol died
- C: ~10% of cells in 2 drops ethanol died
- Cell death increases as ethanol concentration increases

Linear Relationship Between Absorbance and Concentration

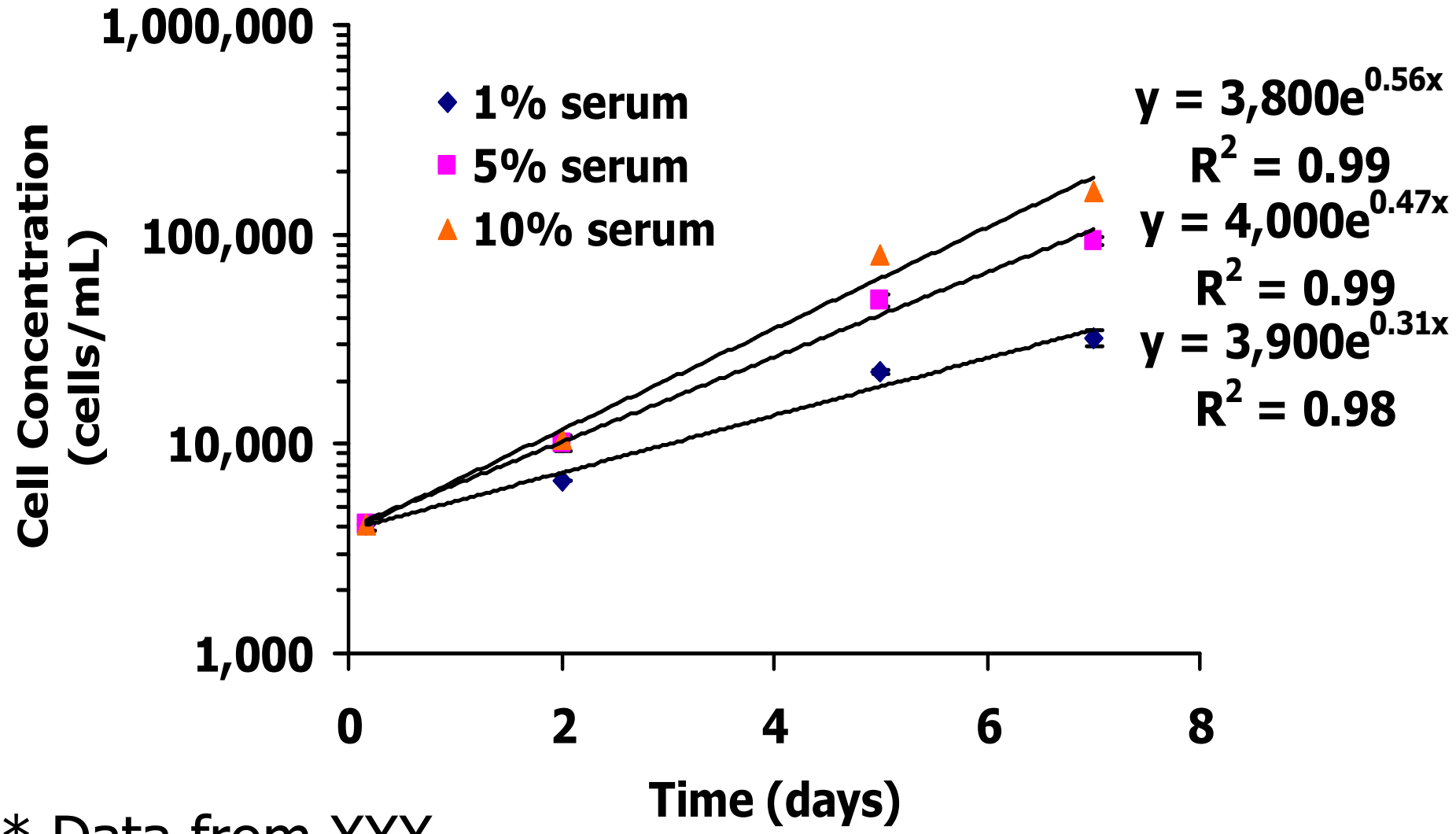
- Increase in cell concentration increases MTT reduction to formazan and thus absorbance
- Absorbance = 3e-5 x [cell] + .0288



Merge MTT and Live/Dead Assays to Improve Viability Assessment

- MTT assay measures relationship between *total* cell concentration and absorbance
- Live/Dead fluorescence assay calculates % viable cells
- Combined, assays can determine relationship between *viable* cell concentration and absorbance
  - $[Cell]_{total}$ x % Viability = $[Cell]_{viable}$
  - Plot Absorbance vs. $[Cell]_{viable}$
  - Find linear fit
- Use relationship to assess toxicity of substances by measuring absorbance

Cell Growth in Different FBS Concentrations*



* Data from XXX

Serum Promotes Cell Growth

- Cell doubling time decreases as serum concentration increases

Serum Concentration	Doubling Time (days)
1%	2.2
5%	1.5
10%	1.2

- Final cell concentration significantly greater in 10% serum than 5% and in 5% than 1% (ANOVA, Tukey, $p < .05$)

Discussion

- Ethanol is highly toxic to HDF cells
- Cell doubling time inversely related to serum concentration
- Relationship between absorbance and viable cell concentration can determine:
  - Cell viability in various growth conditions such as % serum, media type, and temperature
  - Toxicity of drugs in preclinical trials